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Evaluation of depuration procedures to mitigate the off-flavor compounds geosmin and 2-methylisoborneol from Atlantic salmon *Salmo salar* raised to market-size in recirculating aquaculture systems

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ABSTRACT

Fish cultured within water recirculating aquaculture systems (RAS) can acquire "earthy" or "musty" off-flavors due to bioaccumulation of the compounds geosmin and 2-methylisoborneol (MIB), respectively, which are produced by certain bacterial species present in RAS biosolids and microbial biofilms. Fish cultured in RAS are generally transferred to separate depuration systems that are flushed with water in a single pass or operated with limited water recirculation (with no biofilter), in order to purge these unpalatable flavors. Technologies and standard operating practices that optimize purging kinetics for Atlantic salmon *Salmo salar* and other species cultured in RAS are needed to improve the consistency and efficacy of depuration. A 2×2 factorial trial was conducted to evaluate techniques to mitigate off-flavor from Atlantic salmon cultured to 3–5 kg in a semi-commercial scale freshwater RAS. Twelve replicated depuration systems (0.5 m^3) were used to evaluate four combinations ($n = 3$) of the following standard operating procedure and system design parameters: (1) disinfection of depuration systems as a 1 h static treatment using 250 mg/L hydrogen peroxide (H_2O_2) prior to stocking fish, (2) no disinfection prior to stocking fish, (3) presence of water aeration media within gas transfer columns of depuration systems, and (4) absence of water aeration media within gas transfer columns of depuration systems. Food-size Atlantic salmon were stocked within the depuration systems and kept off feed for 10 days. Six salmon were harvested from the original RAS on Day 0 and fileted for baseline assessment of off-flavor concentrations. Thereafter, filet samples ($n = 3$–4) were taken on Days 3, 6, and 10 to evaluate off-flavor kinetics. Hydrogen peroxide disinfection of depuration systems resulted in significantly reduced off-flavor in salmon filets during the depuration period. Results also indicated that the presence of high-surface-area water aeration media shielded biofilms from complete disinfection, resulting in less and slower off-flavor removal from Atlantic salmon filets; while depuration systems void of media resulted in greater and more rapid off-flavor reduction. Thus, water aeration media should not be used in depuration systems because of the challenges posed for effective cleaning, disinfection, and inactivation of off-flavor producing bacteria that may be present, and unit processes and locations that are difficult-to-clean should be excluded. In addition, a wide range of off-flavor concentrations were measured within individual salmon, indicating that one salmon is not a representative sample size to determine market suitability.

1. Introduction

Land-based, water recirculating aquaculture systems (RAS) offer potential for economical and environmentally sustainable culture of a variety of popular food-fish species, including Atlantic salmon (*Salmo salar*). These production systems provide

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advantages such as control of environmental parameters to optimize fish health and growth, enhanced biosecurity, minimal use of water resources and thus flexibility to locate close to major seafood markets, and low environmental impact (Summerfelt and Vinci, 2008); however, one apparent drawback of RAS is the tendency for the development of off-flavor compounds in the filets of fish cultured within these systems, particularly salmonids (Schrader et al., 2005; Houle et al., 2011; Schrader and Summerfelt, 2010; Schrader et al., 2010; Petersen et al., 2011; Burr et al., 2012). Certain off-flavor compounds can impart a "musty" or "earthy" flavor to the filet which negatively impacts product quality and can result in significant economic consequences (Engle et al., 1995; Tucker, 2000). The two most common compounds known to cause earthy and musty off-flavors in RAS are the naturally produced organic chemicals geosmin and 2-methylisoborneol (MIB) (Avault, 1994; Schrader et al., 2005; Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010; Houle et al., 2011), which are secondary metabolic products of certain species of cyanobacteria and actinomycete bacteria. Actinomycete bacteria, typically found within RAS biosolids and microbial biofilms, are the primary producers of the persistent off-flavor compounds in RAS (Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010).

When fish are exposed to a dissolved chemical or compound, such as geosmin or MIB, within the ambient water, uptake of the compound generally occurs through the gills, gut, or skin and can result in subsequent accumulation within lipid-rich tissues (Spacie and Hamelink, 1985; Howgate, 2004). In the case of geosmin and MIB, uptake is primarily through the gills (Howgate, 2004). Compounds that are absorbed by fish can also diffuse out of the fish upon transfer to a clean water system with a lower concentration of the bioaccumulated chemical (Spacie and Hamelink, 1985; Howgate, 2004). In aquaculture, this physiological process serves as an advantageous mechanism for off-flavor reduction and is referred to as depuration or purging. Currently, the recommended practice to depurate off-flavor from salmonids produced in RAS is to transfer fish to flow-through or partial water reuse systems without biofilters for a period of days to weeks prior to slaughter, during which time fish are kept off-feed (Burr et al., 2012).

Although off-flavor has been described as a common problem for fish cultured within RAS, very little research has been devoted to evaluating depuration system technologies and standard operating procedures to optimize the depuration process for salmonids reared in these systems. Burr et al. (2012) conducted the first trial evaluating purging kinetics for Atlantic salmon cultured in RAS and concluded that 10–15 days of depuration within separate clean, biofilm-free systems was required for the greatest reduction of geosmin and MIB from salmon filets. Burr et al. (2012) also demonstrated that granular activated carbon filtration of the makeup water flow entering depuration systems was beneficial in reducing off-flavor compounds in the culture water and salmon filets. In addition, Acuña-Rubio (2002) found that activated carbon filtration of RAS water was effective in reducing geosmin and MIB concentrations in the recycled flow. Petersen et al. (2011) suggested that geosmin and MIB concentrations in the depuration water must be <10 ng/L in order to effectively purge rainbow trout *Oncorhynchus mykiss*.

Several studies have evaluated methods to mitigate off-flavor within the RAS culture system. Burr et al. (2012) demonstrated that off-flavor concentrations in Atlantic salmon were not always improved when attempting to purge fish within a RAS grow-out system. In addition, Schrader et al. (2010) found that a non-disinfecting dose of ozone did not improve off-flavor in rainbow trout cultured in low-exchange RAS. Prospects for off-flavor reduction within RAS were reported by Guttman and van Rijn (2008) who found that production of off-flavor compounds tended to be reduced within an anaerobic treatment loop of a zero water exchange RAS, and Acuña-Rubio (2002) who determined that aeration/degassing was an effective method for reduction of MIB and geosmin from RAS water. However, more research is necessary to understand and optimize these techniques before they can be implemented as consistent and reliable mechanisms for off-flavor removal in RAS-produced fish. Until technologies and strategies to limit off-flavor producing bacteria directly within RAS are developed, transferring fish to depuration systems with water containing low concentrations of MIB and geosmin remains the most effective management approach.

Due to water resource limitations, depuration systems are generally operated as partial reuse systems, rather than single-pass flow systems, in order to conserve water. Partial reuse systems are often equipped with water aeration columns that are typically purposed to add dissolved oxygen and remove excess carbon dioxide from the reused water flow, thus allowing culture of greater fish biomass (Summerfelt et al., 2000, 2003). Water aeration columns generally contain packing material that breaks up the cascading water flow and facilitates gas transfer. Over the years, the design of water aeration columns and selection of packing media within these columns has been refined and optimized to limit plugging and accumulation of biosolids. However, packing media still provides ample surface area for the attachment and growth of microbial biofilm, which has been implicated as a common substrate that contains actinomycete populations, geosmin, and MIB in RAS (Schrader and Summerfelt, 2010; Houle et al., 2011) and thus a potential location for off-flavor contribution.

Various sources suggest that unit processes within RAS could potentially harbor biofilms and the off-flavor producing bacteria that are responsible for unpalatable flavors in fish. Guttman and van Rijn (2008) reported that geosmin and MIB produced by *Streptomyces* (a type of actinomycete) were associated with organic-rich conditions inside of a drum filter and nitrifying filter within the aerobic treatment loop of a zero-water discharge RAS. Schrader and Summerfelt (2010) reported significant geosmin concentrations produced by actinomycetes within biofilm and biosolids samples collected from various locations within RAS including: the top of fluidized sand biofilters, inside of an outlet pipe of the tank side drain, the submerged surfaces of radial flow settlers, inside of heat exchangers, and the internal walls of drum filters. In addition, Houle et al. (2011) reported that geosmin, likely associated with cyanobacteria, was present in biofilms from the culture tank wall and biofilter of a RAS during a study with Arctic char. Author communication with private salmonid farmers using RAS has indicated that less-than-optimal purging periodically occurs when using partial reuse depuration systems equipped with water aeration columns. Structured media contained within the water aeration columns of these depuration systems was suspected of retaining biofilm that contributed to poor off-flavor removal and in some instances an increase in off-flavor within filets.

To better understand the problem and develop better depuration solutions, a 2×2 factorial trial was conducted evaluating the effectiveness of two practices: (1) hydrogen peroxide (H_2O_2) predisinfection of the depuration system and (2) presence/absence of water aeration media on the depuration kinetics of geosmin and MIB from harvest-size (3–5 kg) Atlantic salmon purged within 12 replicated partial reuse systems. The present study was conducted to obtain information that might lead to refinement of current technologies and standard operating procedures for depuration of RAS-produced Atlantic salmon and other salmonids.

2. Methods

2.1. Atlantic salmon culture

Atlantic salmon used for the depuration trial were a Cascade strain purchased from Icicle Seafoods, Inc. (Rochester, WA, USA). The salmon were received as fertilized eyed eggs and incubated within a Heath-tray-style recirculating hatching system at 7.5 °C. After hatching, the alevins remained in the system until the majority of yolk sac was absorbed. Alevins were then stocked for first feeding in a flow-through system with twelve 0.5 m^3 circular tanks with 13 °C water. Salmon were cultured within the first feeding system until they reached approximately 70 g, at which time they were transferred to a partial water reuse system, described in detail in Summerfelt et al. (2004). The fish were relocated to a semi-commercial scale RAS with a 150 m^3 culture tank when they reached a mean weight of 0.75 kg. Salmon were harvested for the depuration study when they reached 3–5 kg. The grow-out system recirculated 96.9 to 99.8% of the water on a flow basis, as required to maintain water temperature between 15 and 16 °C, which resulted in system hydraulic retention times that ranged from 1.2 to 23 days. The design, unit processes, and water flow direction of the semi-commercial scale RAS are described elsewhere (Davidson and Summerfelt, 2004). Atlantic salmon used for the study were cultured in freshwater throughout their life cycle.

2.2. Experimental design of replicated depuration systems

A 2 × 2 factorial trial was used to evaluate the effectiveness of H_2O_2 pre-disinfection of depuration systems and presence/absence of water aeration media within these systems on concentrations of off-flavor compounds (MIB and geosmin) in Atlantic salmon filets. Twelve identical partial water reuse systems (each with a 0.5 m^3 culture tank) were used as experimental depuration systems. The following treatments, run in triplicate, were evaluated: (1) H_2O_2 disinfection applied and water aeration media present; (2) H_2O_2 disinfection applied and water aeration media absent; (3) no H_2O_2 disinfection and water aeration media present; and (4) no H_2O_2 disinfection and water aeration media absent. Treatments were assigned to each depuration system using a random number generator.

The depuration system design was relatively simple, consisting of a circular culture tank with a bottom, center drain and a PVC water aeration column (1.52-m tall × 0.23-m dia.). Water aeration columns containing media were packed with 1.37-m of individual 5-cm NSW Nor-Pac rings (Jaeger Environmental, Eldorado, KS, USA) (Fig. 1). A 1/8-hp magnetic pump (Model MD-55RLT, Iwaki Co. Ltd., Tokyo, Japan) was used to pump approximately 90 L/min of water from mid-depth of the culture tank and lift it to the top of the corresponding aeration column. Depuration systems were operated with an average makeup water flow rate of 3.8 ± 0.1 L/min (approximately 1 gpm) and thus a 95% recycle rate on a flow basis.

Prior to the study, the depuration systems were intentionally used to culture rainbow trout, in order to create biofilm-coated surfaces and "a worst-case-scenario" for purging. Rainbow trout were removed from the depuration systems one day prior to stocking Atlantic salmon for the depuration trial. At this time, all culture tanks were brushed, but aeration media (present in 6 systems) was not brushed or cleaned. A 35% H_2O_2 solution (Perox-aide™, Eka Chemicals Inc., Marietta, GA, USA) was added to 6 depuration systems to create a target concentration of approximately 250 mg/L, which was recirculated through each system without dilution for 1 h. Target H_2O_2 concentrations were validated using a test kit (Cat. No. 22917-00, Hach Company, Loveland, CO, USA). At the end of the 1-h disinfection period, tanks were drained, refilled, and makeup water flow rates returned to previous settings. The next day 168 Atlantic salmon were harvested from the semi-commercial scale RAS and stocked within the 12 depuration systems, 14 fish per tank. Two extra fish beyond the study requirement were included in case of mortality resulting from handling and transport stress.

Fig. 1. 5-cm Norpac water aeration media used within six experimental depuration systems. Norpac media provides a large amount of surface area for dispersal of water flows and subsequent gas exchange. The increased surface also provides significant area for biofilm attachment.

2.3. Filet sampling and biological data collection

Six salmon were fileted upon removal from the semi-commercial RAS in order to obtain baseline off-flavor concentrations on Day 0. Thereafter, salmon (n = 3–4) were randomly selected from each depuration system and fileted on Days 3, 6, and 10. Skin-off filets were collected, vacuum sealed in individual plastic bags, and frozen. The right-side, anterior third of the filet was collected for each fish. Proximate composition was assayed on salmon filets from the same cohort of fish on two separate occasions, less than one month before (n = 6) and after the study (n = 12) by scientists at the Department of Animal and Nutritional Sciences at West Virginia University (Morgantown, WV, USA). Average values from the two sampling events are reported.

2.4. Water quality analysis

Water quality was monitored in order to maintain similar conditions within each depuration system. Water samples were collected once weekly prior to the study while culturing rainbow trout and on Days 4 and 7 from each depuration system for on-site evaluation of the following: biochemical oxygen demand, total ammonia nitrogen, total phosphorous, and total suspended solids. All water quality parameters were analyzed according to methods described in APHA (2005) and HACH (2003). Dissolved oxygen and water temperature were measured with a handheld YSI probe (Model HQ40d, Hach Company, Loveland, CO, USA).

2.5. Off-flavor measurement techniques

Off-flavor concentrations within Atlantic salmon filet samples were analyzed at the Lacombe Research Centre (Agriculture and Agri-Food Canada, Lacombe, AB, Canada) using a relatively novel technique (Ruan et al., 2013). Standard solutions (100 μg/mL in methanol) of MIB and geosmin and an internal standard of 2-isopropyl-3-methoxypyrazine (100 μg/mL in methanol) were

Table 1
Pre-study water quality concentrations (mg/L) measured while culturing rainbow trout prior to the depuration trail and in-study water quality concentrations measured during the 10-day depuration trial. Data expressed as grand mean ± standard error of the 4 treatment groups.

	Pre-study	In-study
Total suspended solids	3.00 ± 0.24	1.75 ± 0.10
Total phosphorous	0.12 ± 0.01	0.12 ± 0.01
Total ammonia nitrogen	0.36 ± 0.02	0.30 ± 0.06
Biochemical oxygen demand	2.37 ± 0.17	1.22 ± 0.17

purchased from Sigma-Aldrich (St. Louis, MO, USA). The different concentrations of stock standard solution (0.05–500 μg/L) were diluted with methanol and mixed with a saturated NaCl solution to prepare the working standard solutions. Recoveries of MIB and GSM were calculated by spike-in two different concentration standards (30 ng/L and 100 ng/L). All other chemicals were of analytical grade. Commercial stir bars [Twister™] were obtained from Gerstel (Linthicum, MD, USA). Frozen salmon filets were thawed in a cold room (4 °C) for 2–3 h. The thawed salmon filets were cut into small pieces and ground using a Mini-Prep Chopper/Grinder (Cuisinart®, Canada) at high speed for 2 min at room temperature. The ground salmon tissue (≤1 ± 0.08 g) was then put into 10 mL amber vials with 9 mL saturated NaCl solution. The tissue sample in solution was homogenized and extracted simultaneously via stir bar at a spin rate of 1000 rpm at room temperature for 2 h. Then, the stir bar was removed and cleaned by Millipore water twice, and air dried on a lint-free tissue. Two stir bars were placed in a glass thermal desorption tube for Thermal desorption-gas chromatography–mass spectrometry (TD-GC–MS) analysis. The stir bar was thermally desorbed by a programmed thermal desorption unit. Injection was performed in the programmable temperature vaporization solvent vent mode and gas chromatography was carried out on a HP-5 ms fused-silica capillary column [30 m (length) × 250 μm (I.D.) × 0.25 μm (film thickness); Agilent Technologies, Mississauga, ON, Canada]. The oven temperature was programmed from 50 °C (held for 1 min) to 150 °C (held for 1 min), then to 280 °C (held for 0.8 min) at 25 °C/min. Helium was used as the carrier gas at a flow rate of 1.2 ml/min. The mass spectrometer (5975 C, MSD, Agilent Technologies, Mississauga, ON, Canada) was operated in the full scan mode.

2.6. Statistical analysis

Experimental depurations systems within each treatment were used as units of replication for calculation of mean salmon off-flavor concentrations. A two-way ANOVA was used for analysis of off-flavor results, where H_2O_2 and presence/absence of water aeration media were assigned as independent variables and off-flavor concentrations (MIB and geosmin) as the dependent variables. A one-way ANOVA and Tukey's post hoc analysis were employed to evaluate statistical differences between treatments and within treatment over time. Normality was assessed using a Shapiro–Wilk test. A probability value (α) of 0.05 was utilized for all analyses.

3. Results

3.1. Background information

Similar water quality was maintained amongst depuration systems prior to the study while culturing rainbow trout in the same systems and during the 10-day study period (Table 1). Establishment of similar water quality conditions was critical so that microbial communities influencing off-flavor could persist in each depuration system. During the depuration trial, dissolved oxygen was maintained near saturation, at 9–10 mg/L in each depuration tank, and water temperature was maintained between 13 and 14 °C. Proximate composition analysis of salmon filets harvested directly from the semi-commercial RAS indicated the following: moisture = 62.9 ± 0.90%; crude fat = 15.9 ± 1.08%; crude protein = 20.2 ± 0.34%; and ash = 1.16 ± 0.02%.

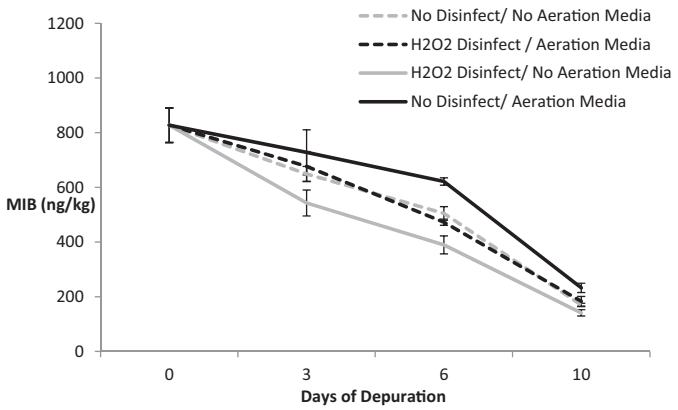

Fig. 2. Methylisoborneol (MIB) concentrations (ng/kg) in Atlantic salmon (filets) purged over a 10-day period within depuration systems with various treatments applied. Data presented as mean ± one standard error.

3.2. MIB levels in salmon filets

Mean concentrations of MIB in the salmon filets declined significantly for all treatments by Day 10 of the study period. Mean MIB concentrations dropped or were unchanged, depending on treatment regimen, over each incremental sampling period (Days 0, 3, 6, and 10), with lowest mean MIB concentrations of 141.0 ± 11.6 ng/kg measured within depuration systems that were void of aeration media and pre-treated with H_2O_2 (Fig. 2). The greatest mean MIB concentrations were measured in salmon that had been held within depuration systems that were not disinfected with H_2O_2 and also contained water aeration media (Fig. 2). Two-way analysis of variance indicated that H_2O_2 disinfection of depuration systems resulted in significantly lower concentrations of MIB on Days 6 and 10 of the study (P = 0.001and 0.008, respectively). In addition, the absence of aeration media resulted in significantly lower concentrations of MIB within salmon filets on Days 6 and 10 of the study (P = 0.004 and 0.002, respectively). Factorial analysis indicated that the effects of H_2O_2 and water aeration media on MIB depuration occurred independently and were not necessarily enhanced by combinations of the two treatments (Day 10; P = 0.199).

3.3. Geosmin levels in salmon filets

Geosmin concentration in filets declined significantly for all treatments by Day 10 of the study period (Fig. 3). The greatest mean geosmin concentrations were measured within salmon cultured within depuration systems that were not disinfected with H_2O_2 and also contained water aeration media (Fig. 3). Two-way analysis of variance indicated that H_2O_2 disinfection of depuration systems resulted in significantly lower concentrations of geosmin on Day 6 of the experiment (P = 0.038). However, a significant difference was not detected between treatments relative to H_2O_2 disinfection on Day 10 (P = 0.205). From Days 6 to 10 mean geosmin concentrations in salmon filets did not change for systems disinfected with H_2O_2 (Fig. 3). For example, on Day 6 salmon from depuration systems disinfected with H_2O_2 with and without aeration media had mean geosmin concentrations of 115.6 ± 25.1 and 97.6 ± 4.5 ng/kg, respectively; and on Day 10 salmon from

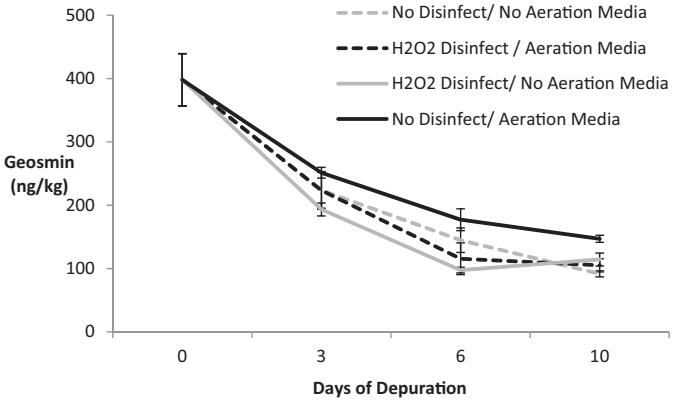

Fig. 3. Geosmin concentrations (ng/kg) in Atlantic salmon (filets) purged over a 10-day period within depuration systems with various treatments applied. Data presented as mean ± one standard error.

the same systems had geosmin concentrations of 105.1 ± 10.3 and 114.4 ± 10.2 ng/kg, respectively (Fig. 3). Two-way analysis of variance also indicated that the absence of packing media within the water aeration columns of the depuration systems caused significantly lower concentrations of geosmin in salmon filets on Day 10 of the study ($P = 0.022$). Factorial evaluation indicated that H_2O_2 disinfection and absence of water aeration media interacted to mitigate geosmin. Overall, the combination of H_2O_2 disinfection of depuration systems and absence of packing media within the water aeration columns of these systems resulted in lower concentrations of geosmin, particularly by Day 6, in comparison to treatments offering the alternatives to these conditions.

3.4. Off-flavor variation in filets

Tables 2 and 3 illustrate that geosmin and MIB concentrations within filets varied substantially amongst individual fish during all sampling events, with the largest degree of variation occurring at Day 0 (the time of harvest from the semi-commercial scale RAS). The wide range in MIB concentration within individual Atlantic salmon filets on Days 6 and 10 of the depuration trial is also illustrated in Fig. 4. After 10 days of depuration, the narrowest range for MIB within individual salmon occurred within depuration systems that were pre-disinfected with H_2O_2 and absent of water aeration media (Fig. 4), i.e. 152 ng/kg as compared to >200 ng/kg for all other treatments (Table 2). Similarly, the tightest range in geosmin concentrations after six days of depuration occurred within depuration systems that were pre-disinfected with H_2O_2 and absent of water aeration media, i.e. 97 ng/kg versus 143–290 for all other treatments (Table 3).

3.5. Statistical differences with time

Tables 2 and 3 also provide notation for statistical differences in off-flavor over time within each treatment group. Fig. 2 illustrates that MIB was significantly lower from Day 0 to Day 3 for depuration systems that were pre-disinfected with H_2O_2 and absent of water aeration media. This was also the only condition in which a significant difference was observed within treatment, following only three days of depuration (Table 2). Conversely, depuration systems that were not disinfected with H_2O_2 and contained water aeration media within the gas transfer columns did not result in a significant reduction in MIB until Day 6 of the depuration period (Table 2). Table 3 illustrates that geosmin was quickly reduced from Day 0 to Day 3 for all treatments with the exception of depuration systems that were not pre-disinfected with H_2O_2 and also contained water aeration media within the gas transfer columns. Geosmin concentrations within salmon cultured within these depuration systems did not decline significantly until Day 10 of the study.

4. Discussion

4.1. Overview of findings

The key study variables (H_2O_2 disinfection and absence of water aeration media) were the most effective at significantly reducing off-flavor in Atlantic salmon filets in comparison to their alternative variables (no H_2O_2 disinfection and presence of water aeration media). The parameters, H_2O_2 disinfection and absence of water aeration media were shown to act independently of each other and therefore had an additive effect for MIB reduction, whereas these variables were found to interact for geosmin reduction.

Off-flavor reduction within depuration systems that were pre-disinfected with H_2O_2 was likely achieved through inactivation of biofilms containing off-flavor producing bacteria. H_2O_2 disinfection was effective at reducing geosmin and MIB in all treatments where it was used; however, significantly lower MIB and geosmin concentrations in salmon filets were achieved for depuration systems without water aeration media. These results suggest that H_2O_2 disinfection was ineffective at complete inactivation of off-flavor-producing bacteria that were associated with biofilms attached to the water aeration media. The Norpac-style water aeration media that was used during the present study provided a large amount of surface area for the attachment of biofilms (Fig. 1). Presumably, biofilm located in certain pinch-points was extremely thick and difficult to completely disinfect with H_2O_2, or a portion of that surface area was shielded to water flowing past it continuously in the same direction. Therefore, H_2O_2 most likely did not contact all biofilm, thereby leaving some biofilms that were not disinfected, which allowed off-flavor producing bacteria to persist. Future research should attempt to identify the off-flavor producing species of bacteria, their location within the depuration system, and the manner in which these bacteria are affected by variables such as type and concentration of disinfectant in order to better understand the mechanisms responsible for off-flavor reduction.

4.2. Refinement of depuration system design

The study results strongly suggest that water aeration media should be excluded from use in gas transfer columns used within depuration systems or alternatively, removed and thoroughly cleaned and disinfected between each depuration event. From a practical perspective, water aeration media is not required within the gas transfer devices of depuration systems. In the absence of water aeration media, gas transfer is reduced (Boyd and Watten, 1989); however, reduced gas transfer efficiency can be compensated for by increasing the water flow passing through the aeration column. In addition, the need for maximum gas transfer efficiency within depuration systems is not as great because fish have lower oxygen demand when kept off feed. Therefore, fish held in depuration systems generally utilize less oxygen and therefore produce less carbon dioxide. Thus, a cascading water flow that does not contact water aeration media can still provide enough gas transfer to support significant fish biomass, as was the case during the present study, where initial average fish densities within each depuration system were approximately 100 kg/m^3. Dissolved oxygen was still maintained near saturation in all depuration systems despite relatively high fish densities. It should be noted that cascading water was not the only means of oxygenation for these systems. A low-head-oxygenation unit provided approximately 150% oxygen saturation within the makeup water entering the depuration systems, but this water flow represented only about 5% of the total

Table 2
MIB range (ng/kg) in Atlantic salmon filets (n = 6 – Day 0; n = 3–4 – Day 3, 6, 10) from individual fish measured over the 10-day depuration period.

Aeration Media Absent	H_2O_2 Disinfection	MIB (ng/kg)			
		Day 0	Day 3	Day 6	Day 10
	√	555–993	538–825	247–697**	100–318†,♦,‡
		555–993	530–1082	492–784	89–365†,♦,‡
√	√	555–993	390–694*	278–588**	74–226†,♦,‡
√		555–993	440–899	366–608**	56–330†,♦,‡

The following notations indicate significant differences across days within treatment:
* Significantly different Day 0–3.
** Significantly different Day 0–6.
† Significantly different Day 0–10.
♦ Significantly different Day 3–10.
‡ Significantly different Day 6–10.

Table 3
Geosmin range (ng/kg) in Atlantic salmon filets (n = 6 – Day 0; n = 3–4 – Day 3, 6, 10) from individual fish measured over the 10-day depuration period.

Aeration media absent	H_2O_2 disinfection	Geosmin (ng/kg)			
		Day 0	Day 3	Day 6	Day 10
	√	265–516	54–323*	67–210**	18–298†
		265–516	106–455	84–234**	76–264†
√	√	265–516	46–319*	61–158**	35–210†
√		265–516	86–327*	33–323**	19–146†

The following notations indicate significant differences across days within treatment:
* Significantly different Day 0–3.
** Significantly different Day 0–6.
† Significantly different Day 0–10.

flow within each systems, thus oxygenation via the makeup water to support fish biomass was negligible [in these partial water reuse systems]. Carbon dioxide concentrations averaged <10 mg/L when the purge systems supported 100 kg/m^3 of unfed fish. Carbon dioxide does not accumulate to harmful levels when aeration of the recirculating flow is only used to meet the oxygen demands of the fish (Speece, 1981).

The simple design of the partial reuse depuration systems used for the present study was advantageous in isolating the potential origin of off-flavors because the systems consisted of minimal piping and only three unit processes: the culture tank, a pump, and a water aeration column. The tanks were thoroughly brushed prior to the study, while the aeration columns and water aeration media (present for 6 depuration systems) were not cleaned. Therefore, biofilm and off-flavor producing bacteria were likely contained within the media of the aeration columns. The results showing lowest off-flavor concentrations within systems without media versus highest off-flavor concentrations with water aeration media present support this conclusion. However, gas transfer columns containing water aeration media are only one of many locations within RAS that could potentially harbor biofilms and attached biosolids that contain off-flavor producing bacteria. Several studies have found that off-flavor compounds, particularly geosmin, were associated with the biofilms and biosolids found within a variety of unit processes throughout RAS including biofilters, drum filters, solids settling devices, heat exchangers, biofilters, and pipes (Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010; Houle et al., 2011). Therefore, it is reasonable to conclude that depuration systems should be relatively simple in design with limited unit processes and hard-to-reach locations that are difficult to clean and/or disinfect. As a starting point in refinement of depuration system design, the results from the present study indicate that water aeration media should be absent from water aeration columns or gas transfer devices. Future research efforts

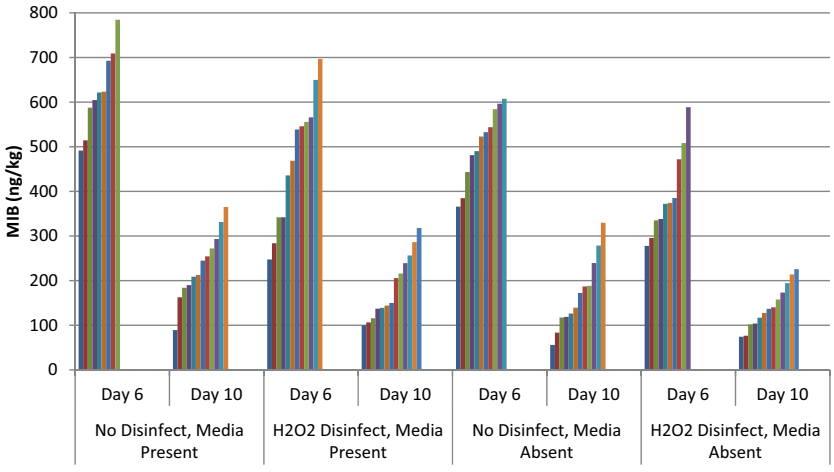

Fig. 4. MIB concentrations measured within filets of individual Atlantic salmon from each of the four depuration treatments on Days 6 and 10. Note that for purposes of data presentation MIB concentrations from all fish used within a treatment are included.

to optimize depuration systems, techniques, and standard operating procedures might include evaluation of the presence/absence of other unit processes or components, as well as optimal H_2O_2 concentrations to mitigate off-flavors.

4.3. Perspective on off-flavor sensory threshold

The presence of analytically detectable concentrations of off-flavor compounds does not mean that fish are off-flavor. The off-flavor concentrations present in salmon filets prior to depuration were relatively low compared to sensory threshold levels for geosmin and MIB in other salmonids. For example, Persson (1980) reported sensory thresholds for geosmin and MIB in rainbow trout of 6500 and 550 ng/kg, respectively, based on 75% recognition by a taste panel. More recently, Robertson et al. (2005) suggested sensory thresholds for geosmin of about 900 ng/kg in rainbow trout filets. The human sensory threshold for geosmin and MIB-related off-flavors is not necessarily equal amongst species. Recent studies have indicated that the human sensory threshold for MIB in Atlantic salmon filets could be >900 ng/kg and approximately 400–450 ng/kg for geosmin (Burr et al., 2012). However, finite establishment of sensory off-flavor thresholds was not the primary objective of the Burr et al. study; therefore, more research is needed to define the off-flavor threshold to the human palate for RAS-produced salmon filets. Initial geosmin and MIB concentrations in the Atlantic salmon harvested directly from the semi-commercial scale RAS in the present study ranged from 265 to 516 ng/kg and 555 to 993 ng/kg, respectively; therefore, the concentrations of these compounds in most fish at the initiation of this study were possibly below the minimum detection limit of the human palate. Although, the initial off-flavor concentrations were relatively low, MIB and geosmin concentrations were still reduced 4- to 5-fold from Day 0 to 10 of the depuration period. Based on this substantial reduction, similar depuration kinetics could be expected for salmon with greater original off-flavor concentrations. This is supported by Robertson et al. (2005) which showed that depuration kinetics were not drastically different for three groups of rainbow trout with various initial off-flavor concentrations of approximately 1.6, 3.0, and 6.2 μg/kg, when the fish were purged under similar conditions.

The relatively low initial off-flavor concentrations observed during the present study should not lead to the conclusion that Atlantic salmon harvested from semi-commercial scale RAS do not require relocation to odor-free depuration systems prior to harvest, for several reasons. One reason is that off-flavor concentrations can vary significantly within the culture water of the same RAS over a relatively short period of time (Burr et al., 2012). Therefore, a population of fish from the same RAS could have adequate flavor one week, but might have unacceptable levels of off-flavor the following week. This phenomenon has not been fully explained, but is most likely related to variation in environmental and/or nutritional factors that impact the ecology of actinomycete populations that are responsible for these off-flavor compounds (Schrader et al., 2013). Another reason is that off-flavor concentrations within fish cultured in RAS can be highly variable within individuals of the same population as was observed during the present study (Tables 2 and 3; Fig. 4). For example, MIB ranged from 74 to 226 ng/kg on Day 10 within individual salmon from depuration systems pre-disinfected with H_2O_2 and absent of water aeration media (Fig. 4), representing the tightest range in MIB amongst individual fish in any treatment. The goal of depuration should be that all fish are adequately purged and below the off-flavor threshold of the human palate. Tasting just one fish is not likely sufficient to ensure that the entire population is on-flavor. The necessity for flavor quality testing was demonstrated in a recent study by Schrader and Tucker (2012) concerning populations of pond-raised channel catfish. A third reason involves the time and expense involved with instrument analysis and verification of flavor quality. Outside of blind tasting, simple instrumentation is not available to validate off-flavor concentrations at the farm. Sending filet samples to laboratories with the appropriate technologies and expertise to measure off-flavor is expensive, and results may not be provided for several weeks; therefore, this would not be a feasible approach for farmers to assess off-flavor. Because of these uncertainties, a standardized depuration process is highly recommended to ensure reduction of MIB and geosmin in RAS-produced fish, including salmonids.

4.4. Time period for optimal depuration

The lowest off-flavor concentrations within Atlantic salmon filets were generally achieved after 10 days of depuration for all treatments. These findings are consistent with those of Burr et al. (2012), who concluded that 10–15 days of depuration was optimal for Atlantic salmon cultured in RAS. The results from the present study indicate some potential for a reduced depuration period (<10 days) when utilizing optimized depuration procedures. The fastest rates of off-flavor reduction were observed in depuration systems that were pre-disinfected with H_2O_2 and void of water aeration media; and the slowest rates of off-flavor depuration occurred in systems that were not pre-disinfected with H_2O_2 and contained water aeration media. Geosmin concentrations appeared to approach a potential lowest achievable threshold by Day 6. Under these circumstances, an optimal depuration period could have been as little as 6 days for geosmin, particularly for treatments for which H_2O_2 disinfection was applied and water aeration media absent. However, based on the study results it is difficult to recommend a definitive length of time for depuration or determine exactly how many days the depuration period might be reduced when using optimized techniques.

Future research investigating optimized depuration techniques should evaluate depuration more frequently during the critical last days, i.e. days 6–10 (present study) and possibly beyond, which could help to define an optimal length of time for depuration. It should be noted that time of depuration can be dependent upon a variety of factors including water temperature, adipose content of the fish, initial off-flavor concentration contained in the filet (Johnson and Lloyd, 1992; Perkins and Schlenk, 1997; Dionigi et al., 2000), and off-flavor concentration of the depuration system supply water (authors' personal experience); therefore, the time period needed for optimal depuration of RAS-produced salmon is likely site-specific depending on these factors. Proximate composition results of salmon used in the present study were included as a reference for filet lipid content. Increased adipose content would likely result in higher concentrations of bioaccumulated geosmin and MIB in the salmon flesh, which would require a longer depuration period.

5. Conclusion

Salmon cultured to food-size in RAS will likely require transfer to separate, odor-free depuration systems that that are flushed with water in a single pass or are operated with limited water recirculation (with no biofilter), in order to purge off-flavors. The findings of this study suggest: (1) pre-disinfection of depuration systems using 250 mg/L H_2O_2 is effective at reducing off-flavor in Atlantic salmon and (2) water aeration media should not be used within depuration systems because of the challenges posed for effective cleaning, disinfection, and inactivation of off-flavor producing bacteria. The authors recommend inclusion of these techniques as standard operating procedures to optimize the depuration process for Atlantic salmon and other salmonids that are cultured to market-size within recirculating aquaculture systems.

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